

Addressing speciation in the effect factor for characterisation of freshwater ecotoxicity—the case of copper

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Abstract

Purpose Determination of the ecotoxicity effect factor (EF) in life cycle impact assessment (LCIA) is based on test data reporting the total dissolved concentration of a substance. In spite of the recognised influence of chemical speciation and physico-chemical characteristics of the aquatic systems on toxicity of dissolved metals, these properties are not considered when calculating characterization factors (CFs) for metals. It is hypothesised that the main cause of the variation in reported EC₅₀ values of Cu among published test results lies in different speciation patterns for Cu in the test media, and that the toxicity of Cu is predominantly caused by the free Cu²⁺ ion. Hence, the free Cu²⁺ ion concentration should substitute the total dissolved metal concentration when determining the EF.

Materials and methods The study was based on a review of published ecotoxicity studies reporting acute and chronic EC₅₀ data for Cu to *Daphnia magna* and to different species of fish and algae. The speciation pattern of Cu in the different media applied in the studies was calculated using the Visual MINTEQ model. EFs were calculated according to the expression applied in the USEtox™ characterization model.

Results and discussion Reported EC₅₀ values for Cu show variations of one to several orders of magnitude for the same organism, but the study indicates that the large variation is caused by differences in water chemistry of the test media influencing the metal speciation. The relationship between the calculated free Cu²⁺ ion concentration and reported EC₅₀ values indicates that the aquatic ecotoxicity of Cu to *D. magna* can be predicted from the free ion concentration. Other results confirm that the free Cu²⁺ ion concentration depends on the [Cu]/[DOC] ratio since the majority of the total dissolved Cu is present as Cu-DOC complexes when the media contains more than 1 mg/L of DOC, and since Cu in such complexes has limited availability to the test organisms.

Conclusions These results suggest that speciation should be taken into account in the modelling of both EFs and fate factors for LCIA, and the EF for Cu in the aquatic environment should be based on the concentration of the free Cu²⁺ ion.

Keywords Copper speciation · *Daphnia magna* · DOC · Effect factor · Free Cu²⁺ ion · Life cycle impact assessment

1 Introduction

For ecotoxic impacts, the characterization modelling in life cycle impact assessment (LCIA) expresses the relative hazard of a chemical in a characterisation factor (CF) that is determined as the product of a fate factor (FF) and an effect factor (EF).

$$CF_{j,i,s} = FF_{j,i,s} \cdot EF_{j,s} \quad (1)$$

Where CF_{j,i,s} (potentially affected fraction (PAF) m³ day kg⁻¹) is the compartment-specific environmental charac-

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terisation factor of a substance s emitted to compartment i and transported to compartment j . The fate factor $FF_{j,i,s}$ (d) typically expresses the mass of substance s in compartment j resulting from the emission to compartment i , while the effect factor $EF_{j,s}$ ($PAF\ m^3\ day\ kg^{-1}$) expresses the ecotoxic effect that can be expected in compartment j as a result of an increase in the amount of substance s . In recognition of the influence that speciation has on the environmental fate of metal compounds, several studies have addressed how to include this in the calculation of FF for metals, e.g. Bhavsar et al. (2004), Bhavsar et al. (2008) and Strandesen et al. (2007).

The bioavailability and the toxicity of a metal depend on its distribution between different chemical forms, but in spite of the recognised influence of chemical speciation and physico-chemical characteristics of the aquatic systems on metal toxicity, these properties are not considered when calculating CFs for potentially toxic metals. The free metal ion concentration has earlier been reported to be a good predictor of ecotoxicity in freshwaters (Campbell 1995). It was first recognised by Steeman-Nielsen and Wium-Andersen (1970) that free Cu^{2+} ions are very toxic to algae even at the low levels found in natural waters. Later it was realised that the amount of Cu required to give a biological response like toxicity or reduction of growth in natural waters is different depending on the water chemistry (Allen 2000). For some metals, biotic ligand models (BLMs) have been developed that include modelling of the metal's speciation in the environmental medium and of the interaction with the biotic ligand, e.g. the gill (in the case of fish) to give a more accurate prediction of the toxicity. The BLMs are based on the knowledge of water chemistry and chemical speciation in the water and on the biotic ligand (Pagenkopf 1983) and are considered state-of-the-art within metal toxicity modelling. So far, however, BLMs have only been developed for a few metals and hence do not represent an option for use in LCIA where a consistent characterisation is needed for a wider group of metals. In order to consider the influence of speciation on ecotoxicity, some other approach must be taken.

Since LCA is focused on comparisons, the CFs applied in LCIA must express the potency of different chemicals on a mass basis relative to each other. Particularly for the comparison between metal compounds and organic chemicals, this poses a serious challenge (Ligthart et al. 2004), and while some consensus has been reached on the assessment of freshwater ecotoxic impacts of organic chemicals (Hauschild et al. 2008; Rosenbaum et al. 2008), the assessment of metals in LCIA is still a field of fundamental developments (Diamond et al. 2010).

Traditionally, reported ecotoxicity measures like EC_{50} values for the effects of metals in aquatic systems are based on total metal concentrations (e.g. De Schampelaere et al.

2007; Heijerick et al. 2005). The environmental fate and effect of metals differ from those of organic compounds in several aspects. Apart from the fact that organic pollutants degrade and metals are non-degradable elements, an important difference resides in the fact that metals speciate and the bioavailability of the metal varies strongly among species. While the use of total dissolved concentrations may be justified for non-dissociating organic pollutants, the toxicity of metals in water thus depends on the concentrations and activities of specific chemical forms (species) of the metal rather than on the total dissolved concentrations (e.g. Heijerick et al. 2003; Strandesen et al. 2007). The speciation pattern of metals is influenced by the overall composition of the solution (synthetic or natural), in particular, properties like pH, hardness, ionic strength and the presence of complexing ligands such as inorganic ions, EDTA (in synthetic test media) and naturally occurring dissolved organic matter (DOM) expressed as dissolved organic carbon (DOC). The water chemistry and resulting speciation of the metal in the test media thus strongly influence the bioavailability and hence also the ecotoxicity of the metal (Bossuyt et al. 2004; Bury et al. 1999; Hollis et al. 2000; Kramer et al. 2004; Macdonald et al. 2002; Richards et al. 2001). The distribution of the metal among the various metal species, and the resulting concentrations of each of them can be predicted by chemical speciation models (Bhavsar et al. 2004), but in spite of the recognised influence on the metal ecotoxicity, chemical speciation and physico-chemical characteristics of the aquatic system are rarely considered when reporting on ecotoxic effects for metals and deriving water quality criteria (Janssen et al. 2000). Water quality standards for trace elements are traditionally defined for dissolved and total metal concentration and based on laboratory tests with synthetic test solutions which do not reflect the availability of the metal in natural waters (Janssen et al. 2000).

The speciation also influences the ecotoxicity of the metal ion, and must hence be respected in the calculation of the EF as well in order to arrive at consistent CFs, and recent studies have shown that integration of speciation and bioavailability by introducing BLM in the characterization modelling for ecotoxicity in LCIA is a better representative than total dissolved concentrations of metals (Gandhi et al. 2010; Gandhi et al. 2011). In this study, it is hypothesised that the main cause of the variation in reported EC_{50} values of Cu among published test results lies in different speciation patterns for Cu in the applied ecotoxicity test media, and that the toxicity of Cu is predominantly caused by the free Cu^{2+} ion. In order to test the hypothesis the Visual Minteq version 2.60 is chosen for speciation modelling applied due to its applicability to a wide range of metals. If the hypothesis is proven correct, we will derive corresponding EC_{50} values expressed for the free Cu^{2+} ion

species in the applied test media and calculate the EF for the free Cu²⁺ ion taking the speciation of Cu in the aquatic environment into account. The testing of the hypothesis is done on published data on the ecotoxicity of Cu to crustaceans, algae and fish in freshwater. Cu was chosen due to the relatively high number of studies published on this metal.

2 Materials and methods

2.1 Review and collection of data

A review was performed of ecotoxicity literature studies reporting acute and chronic EC₅₀ data for Cu, to *D. magna* applying a total of 80 different synthetic or natural media. For each study, the reported information on media properties in terms of pH, ionic strength and the concentrations of organic and inorganic ions and ligands was collected together with the reported toxicity in terms of EC₅₀ values. Among the possible ecotoxicity indicators, EC₅₀ values were chosen as they are the recommended basis of EF calculations within LCIA (Hauschild and Pennington 2002). In addition, in order to allow calculation of an EF for Cu, studies of Cu ecotoxicity to algae and fish were reviewed, but the main focus has been on *D. magna* as a keystone species in the freshwater food web, widely used in aquatic ecotoxicity testing (Barata et al. 1998; Geffard et al. 2008).

2.2 Chemical speciation calculations

The speciation pattern of Cu in the different test media was calculated using a chemical speciation model determining the concentrations of the free Cu²⁺ ion as well as of the various dissolved organic and inorganic Cu complexes. A non-commercial software package: Visual Minteq version 2.60 (Gustafsson 2007) was applied to calculate the equilibrium concentration and relative distribution of dissolved Cu between the free Cu²⁺ ion and individual inorganic and organic complexes for each test medium. Since the type of dissolved organic carbon applied in the media is insufficiently specified in all cases, the NICA-Donnan DOC (Kinniburgh et al. 1999) was assumed representative, and the NICA-Donnan model of the Visual Minteq software was used for assessment of metal-organic complexation (Gustafsson 2007).

2.3 Calculation of the EF

The EF was calculated using the formula from the USEtox™ characterisation model (Rosenbaum et al. 2008):

$$EF_{i,s} = 0.5 / HC_{50s} \quad (2)$$

Where EF_{i,s} is the effect factor for substance s for compartment i (PAF m³ kg⁻¹) and HC_{50s} is the hazardous concentration of substance s at which 50% of the species in the ecosystem are exposed above their chronic EC₅₀ concentration (kg m⁻³) (Larsen and Hauschild 2007; Rosenbaum et al. 2008). Two different ways of determining the HC_{50s} have been proposed. First, the geometric mean of reported EC₅₀ values is calculated for each test organism. Then the HC₅₀ value is calculated: (1) as the geometric mean of these mean EC₅₀ values across all test organisms or (2) as the geometric mean of the three geometric means calculated for the mean EC₅₀ values across test organisms from each of the three trophic levels, algae, crustaceans and fish (GM_{troph}) separately. GM_{troph} is claimed to be more representative for the true HC₅₀ of an ecosystem (Larsen and Hauschild 2007). Both approaches were tested, and obvious outliers were taken out prior to the calculations.

3 Results

3.1 Media composition and patterns of Cu speciation

The composition of the selected media is presented in Table 1 for *D. magna* and Table 2 for fish and algae. The synthetic media and natural waters are described in terms of pH and concentrations of organic and inorganic ligands. The concentrations of major cations apart from Cu are not shown but included in the Visual Minteq calculations. The resulting Cu speciation pattern is reported as the distribution of the total dissolved Cu on three fractions: free metal ion [Cu²⁺], total inorganic complexed fraction (sum of all inorganic Cu complexes, i.e. carbonato, sulfato, hydroxy, chloro complexes) and total organic complexed fraction (sum of all organic Cu complexes) (see Tables 1 and 2).

The studies summarised in Tables 1 and 2 cover 80 different media compositions for *D. magna*, 12 media compositions for algae species and 22 for fish species with pH values ranging from 5.5 to 8.8. The ion activity for Cu²⁺ is calculated for each media composition and expressed in concentration units (µg/L) (since the ionic strength is relatively low in all media (<0.03 mol/L) and inventory results in LCA are reported as masses). The calculated speciation patterns show large variations depending on the media composition. The free Cu²⁺ ions vary within the range of 0.01–79.4% of the total dissolved Cu. All media with DOC contain less than 19% free Cu²⁺ ions. The total concentration of inorganic Cu complexes such as CuOH⁺, Cu(OH)₂, CuCO₃⁰ and CuSO₄⁰ range from <1% to 99% of total dissolved Cu; as expected, the highest percentages occur in media without or very low in DOC. The fraction of Cu bound in organic complexes is generally high (more

Table 1 Media composition (selected parameters) for *D.magna* and Cu speciation as calculated using Visual Minteq

Test method	pH	Ion strength	Reported EC ₅₀	Calculated speciation% of total dissolved Cu	Ligands (mg/L)						EC ₅₀ free ion Cu ²⁺ (µg/L)	Reference
					Free Cu ²⁺ (µg/L)	Inorganic	Organic	DOC	Cl [−]	CO ₃ ^{2−}	NO ₃ [−]	
Acute	8.1	9.5E-03	25.0	2.3	97.7	0.0	0.0	175.0	0.0	0.0	0.6	LeBlanc (1982)
Acute	7.2	6.0E-04	21.5	7.5	12.7	79.8	1.0	21.7	0.0	0.0	1.6	Hickey and Vickers (1992)
Acute	7.7	1.7E-02	197.0	1.4	43.7	54.9	5.0	144.9	269.1	0.2	48.4	2.7
Acute	8.3	2.7E-03	130.0	1.5	63.4	35.0	1.1	1.5	82.0	0.0	4.3	2.0
Acute	6.9	9.8E-03	10.9	77.7	22.3	0.0	0.0	215.6	5.6	0.0	24.0	8.5
Acute	6.8	2.7E-03	6.7	76.1	23.9	0.0	0.0	20.6	6.5	0.0	48.0	5.1
Acute	6.8	1.7E-02	21.2	79.4	20.6	0.0	0.0	553.0	5.6	0.0	24.0	16.8
Acute	7.9	3.8E-03	52.4	3.2	96.8	0.0	0.0	50.6	104.4	0.0	24.0	1.6
Acute	7.7	5.8E-03	15.4	4.5	42.9	52.5	0.5	1.9	37.9	0.2	72.4	0.7
Acute	8.1	1.1E-02	119.0	2.1	85.9	12.0	0.4	4.1	76.9	0.2	137.0	2.5
Acute	5.7	5.0E-04	77.3	18.8	0.3	80.8	6.3	5.8	0.0	0.0	1.8	14.5
Acute	5.7	6.0E-04	66.8	15.8	0.3	83.9	6.3	4.4	0.0	0.0	1.4	10.6
Acute	6.9	4.8E-03	128.0	6.5	1.8	91.7	4.5	144.2	0.0	0.0	48.0	8.3
Acute	7.0	4.4E-03	638.0	0.8	0.3	98.9	11.7	146.0	0.0	0.0	49.0	5.3
Acute	7.0	4.7E-03	332.0	0.2	0.1	99.7	10.8	142.1	0.0	0.0	49.0	0.7
Acute	6.9	5.9E-03	53.8	2.1	0.6	97.4	2.0	218.1	0.0	0.0	48.1	1.1
Acute	7.0	6.2E-03	275.0	0.4	0.1	99.5	9.2	226.1	0.0	0.0	48.0	1.1
Acute	7.1	5.4E-03	60.6	1.5	0.7	97.8	2.6	162.4	0.0	0.0	84.8	0.9
Acute	8.4	2.8E-03	49.5	2.2	97.8	0.0	0.0	42.0	0.0	0.0	0.1	Koivisto et al. (1992)
Acute	8.6	8.1E-03	43.1	1.5	17.1	81.4	0.9	27.3	0.0	0.0	141.2	0.7
Acute	8.2	4.1E-03	323.3	4.4	17.3	78.2	0.7	6.7	0.0	0.0	82.9	1.4
Acute	8.4	3.3E-03	48.2	2.0	13.9	84.0	1.1	4.4	0.0	0.0	45.7	1.0
Acute	8.2	1.8E-03	32.0	3.2	13.2	83.6	0.8	2.3	0.0	0.0	17.9	1.0
Acute	8.2	1.8E-03	24.3	2.2	9.1	88.8	0.8	3.5	0.0	0.0	24.7	0.5
Acute	8.6	3.4E-03	56.9	1.9	23.2	74.9	1.0	7.2	0.0	0.0	13.2	1.1
Acute	8.4	3.0E-03	59.9	3.4	23.4	73.3	1.0	2.8	0.0	0.0	36.2	2.0
Acute	8.5	3.1E-03	66.2	0.9	8.2	90.9	1.7	4.0	0.0	0.0	30.8	0.6
Acute	8.2	2.6E-03	12.1	3.6	14.3	82.1	0.4	5.5	0.0	0.0	42.7	0.4
Acute	7.9	2.4E-03	12.1	10.3	19.7	70.0	0.3	5.9	0.0	0.0	45.0	1.2
Acute	8.1	1.0E-03	27.8	1.1	3.7	95.2	1.2	1.5	0.0	0.0	8.3	0.3
Acute	8.4	2.2E-03	28.3	10.8	76.9	12.3	0.1	1.9	0.0	0.0	18.8	3.1
Acute	7.4	6.0E-04	118.5	0.3	0.3	99.4	8.5	8.0	0.0	0.0	8.4	0.4
Acute	8.4	6.7E-03	239.8	1.9	12.5	85.6	3.9	0.9	0.0	0.0	140.9	4.6
Acute	8.2	2.4E-02	314.0	0.01	9.2	90.8	17.3	194.0	577.0	0.0	185.0	0.1
Acute	8.4	1.7E-02	207.0	0.01	2.4	97.5	14.2	310.0	239.0	0.0	94.0	0.01

Acute	8.1	8.4E-03	304.0	0.1	5.9	94.0	14.7	59.0	189.0	0.0	59.0	0.3
Acute	6.6	3.0E-03	10.0	14.9	2.9	82.1	1.5	31.0	8.8	0.0	32.0	1.5
Acute	8.2	8.3E-03	87.0	0.1	8.2	91.7	5.0	82.0	164.0	0.0	51.0	0.1
Acute	8.2	5.9E-03	93.0	0.1	7.5	92.4	5.3	29.0	148.0	0.0	38.0	0.1
Acute	7.8	1.4E-03	60.0	10.9	89.1	0.0	0.0	1.2	27.2	0.0	0.0	6.6
Chronic	6.8	9.0E-03	120.0	2.9	0.6	96.5	10.0	143.5	0.0	0.0	48.0	3.5
Chronic	7.6	8.3E-03	97.0	2.1	10.8	87.1	4.0	142.7	45.0	0.0	48.0	2.0
Chronic	8.1	5.5E-03	16.1	1.8	98.2	0.0	0.0	0.0	135.0	0.0	0.0	0.3
Chronic	7.8	1.4E-03	35.0	7.4	92.6	0.0	0.0	1.2	27.0	0.0	0.0	2.6
Chronic	6.1	8.8E-03	45.8	6.5	0.4	93.2	5.6	274.8	27.2	0.0	19.4	3.0
Chronic	7.9	2.0E-02	115.0	0.4	5.9	93.7	6.3	469.0	72.0	0.0	77.0	0.5
Chronic	7.1	3.4E-02	316.0	1.0	0.5	98.5	21.6	1326.5	14.8	0.0	48.6	3.0
Chronic	7.0	8.9E-03	34.6	7.8	5.0	87.2	2.3	163.8	14.0	0.0	48.0	2.7
Chronic	7.0	1.8E-02	156.0	2.2	1.2	96.6	11.9	392.0	13.6	0.0	96.0	3.4
Chronic	7.0	1.3E-02	250.0	0.9	0.5	98.5	20.1	325.2	13.8	0.0	48.1	2.3
Chronic	6.2	4.7E-03	41.5	7.1	0.6	92.3	5.0	97.0	2.7	0.0	32.6	2.9
Chronic	7.9	1.8E-02	216.0	0.1	1.5	98.3	15.6	344.8	68.4	0.0	118.1	0.3
Chronic	7.1	1.1E-02	138.0	1.7	1.3	97.0	10.3	220.2	15.2	0.0	75.3	2.4
Chronic	7.9	3.2E-02	195.0	0.01	0.8	99.2	18.1	927.5	81.0	0.0	77.3	0.1
Chronic	7.1	2.6E-02	152.0	2.8	1.7	95.5	10.0	668.5	15.2	0.0	96.0	4.2
Chronic	6.1	1.0E-02	122.0	2.2	0.1	97.7	16.9	211.8	2.3	0.0	19.3	2.7
Chronic	7.9	8.9E-03	276.0	0.2	3.3	96.6	13.5	161.0	74.4	0.0	55.1	0.5
Chronic	7.2	1.1E-02	144.0	1.5	1.5	97.0	10.2	223.1	18.0	0.0	75.2	2.2
Chronic	7.1	1.3E-02	263.0	1.4	1.1	97.5	16.1	268.2	15.9	0.0	90.8	3.8
Chronic	5.6	1.0E-02	70.6	11.7	0.9	87.4	9.0	213.9	1.0	0.0	72.0	8.3
Chronic	6.1	2.1E-02	99.4	1.5	0.1	98.4	16.9	723.6	2.4	0.0	19.7	1.5
Chronic	7.0	1.1E-02	48.3	13.4	7.7	78.9	2.1	238.1	13.4	0.0	48.1	6.5
Chronic	7.1	1.9E-02	144.0	2.1	1.3	96.6	9.8	532.1	14.9	0.0	48.3	3.0
Chronic	8.4	2.3E-02	152.0	0.01	2.5	97.4	10.2	546.2	201.6	0.0	48.3	0.01
Chronic	6.1	4.8E-03	49.0	6.3	0.4	93.3	6.2	116.4	2.4	0.0	19.2	3.1
Chronic	7.9	1.6E-02	108.0	0.5	7.1	92.4	5.7	282.0	72.6	0.0	76.8	0.5
Chronic	7.9	1.9E-02	195.0	0.1	0.9	99.1	17.8	393.7	76.8	0.0	76.9	0.1
Chronic	7.0	1.2E-02	192.0	2.1	1.1	96.8	12.8	262.5	12.5	0.0	48.0	4.0
Chronic	8.4	1.6E-02	157.0	0.01	2.5	97.5	11.2	247.2	177.0	0.0	48.0	0.01
Chronic	6.2	7.3E-03	152.0	3.2	0.3	96.5	14.5	170.6	2.7	0.0	57.6	4.8
Chronic	7.8	1.6E-02	84.4	0.5	5.9	93.6	5.2	269.2	66.0	0.0	90.4	0.4
Chronic	7.2	9.3E-03	49.7	13.2	13.8	73.0	1.7	157.8	18.4	0.0	52.6	6.5
Chronic	7.2	1.9E-02	182.0	2.8	2.5	94.7	10.1	365.3	17.9	0.0	122.9	5.1
Chronic	8.3	1.5E-02	145.0	0.01	1.6	98.4	12.3	223.4	167.4	0.0	75.4	0.01
Chronic	6.1	1.3E-02	57.0	18.5	1.9	79.7	4.8	266.7	2.2	0.0	89.6	10.5

Table 1 (continued)

Test method	pH	Ion strength	Reported EC ₅₀	Calculated speciation% of total dissolved Cu (µg/L)	Ligands (mg/L)				EC ₅₀ free ion Cu ²⁺ (µg/L)	Reference			
					Free Cu ²⁺	Inorganic	Organic	DOC	Cl [−]				
Chronic	6.1	1.6E-02	122.0	6.6	0.8	92.6	13.2	331.3	2.2	0.0	112.3	8.1	De Schampelaere and Janssen (2004a, b)
Chronic	7.9	6.7E-03	154.0	0.9	15.8	83.3	4.8	96.5	73.2	0.0	32.0	1.3	De Schampelaere and Janssen (2004a, b)
Chronic	7.1	4.5E-03	166.0	1.1	0.9	98.0	9.1	86.9	15.0	0.0	29.0	1.8	De Schampelaere and Janssen (2004a, b)
Chronic	7.1	1.1E-02	145.0	1.9	1.5	96.6	10.0	221.7	15.5	0.0	74.7	2.8	De Schampelaere and Janssen (2004a, b)
Chronic	8.1	1.0E-02	16.1	0.01	0.66	99.3	99.3	133.0	294.0	0.0	78.0	0.01	VanLeeuwen et al. (1988)
Chronic	7.7	1.7E-02	79.8	2.6	73.3	24.2	5.0	144.9	269.1	0.0	48.4	2.0	Bossuyt and Janssen (2003)

Major cations are not included in the table, but they are included in the speciation calculations performed with the model Visual Minteq version 2.60. Acute is 48 h and chronic is 21 days

than 70%) except for a few studies, where the DOC content is very low (<0.1 mg/L) and the content of inorganic ligands like CO₃^{2−} and SO₄^{2−} is high. On the basis of the speciation calculations, the calculated EC₅₀ for the free Cu²⁺ ion (i.e. the free Cu²⁺ concentration corresponding to the reported EC₅₀ value) is shown (see Tables 1 and 2).

Figure 1 shows the calculated fraction of dissolved Cu present in the form of Cu-DOC complexes (as percentage of total dissolved Cu in the media) as function of the DOC concentration in the media. Nine outliers from the general trend in the graph (marked by open circles) seem to be caused by a high test media content of other anionic ligands such as CO₃^{2−} competing with DOC, and of cations such as Fe²⁺ and Al³⁺ competing with Cu²⁺ for the DOC binding sites. The Cu(II)-DOC species concentration is determined for all media using the same complexing constants for DOC disregarding potential, but unknown, differences in the composition of the DOC. This is a fair assumption as the Nica-Donnan model used in Visual Minteq is created by combining the NICA isotherm with the Donnan model to describe counter-ion accumulation. The NICA isotherm enables simulation of cation complexation to constituents that are highly heterogenous with respect to binding affinity (e.g. humic and fulvic acids) (Kinniburgh et al. 1999; Tipping 1998). Accordingly, DOC from different sources has earlier been found to affect Cu toxicity to *D. magna* in the same way (De Schampelaere and Janssen 2004b), although some studies have found that minor differences between DOCs from different sources can be expected and will result in differences in the protolytic properties, e.g. an investigation of several hundred streamwater samples from Sweden and the Czech Republic in Hruska et al. (2003).

3.2 Speciation and Cu toxicity (EC₅₀)

Figures 2, 3, 4, 5 present the relationship between the calculated concentrations of different Cu species in the applied test media and reported *D. magna* EC₅₀ values in the studies. The test media comprise both natural waters and synthetic test media, but the speciation reveals no consistent differences in the relationship between the predicted [Cu²⁺] and the reported EC₅₀ values for synthetic and natural media.

Figures 2 and 3 show the reported acute and chronic EC₅₀ values for Cu as function of the calculated concentrations of different classes of Cu species in the test media. With the concentration of organic or inorganic Cu complexes, both acute and chronic-reported EC₅₀ values form a nearly linear relationship with the Cu species concentration (R^2 , acute=0.959, chronic=0.981). Cu complexes thus seem to have a low bioavailability for *D. magna*, although some studies (Tandy et al. 2006; Tao et al. 2000) have

Table 2 Media composition (selected compounds) for fish and algae and Visual Minteq calculated Cu speciation

Test method	Organism	Species	pH	Reported EC ₅₀ (µg/L)	Calculated speciation% of total dissolved Cu			Ligands (mg/L)			EC ₅₀ free ion Cu ²⁺ (µg/L)	Reference	
					Free Cu ²⁺	Inorganic	Organic	DOC	Cl ⁻	CO ₃ ²⁻	SO ₄ ²⁻		
72 h	Algae	Green algae (<i>Pseudokirchneriella subcapitata</i>)	7.8	16.5	1.5	3.0	95.4	<1	26.2	0.0	6.7	0.3	Heijerick et al. (2005)
24 h	Algae	<i>P. foveolarum</i> Gom.	7.6	19.0	0.7	99.3	0.0	0.0	0.1	0.0	40.0	0.1	Takamura et al. (1990)
24 h	Algae	<i>P. ramosum</i> Boye-Pet	7.6	53.0	0.7	99.3	0.0	0.0	0.1	0.0	40.0	0.4	Takamura et al. (1990)
24 h	Algae	<i>P. unicinatum</i> Gom.	7.6	13.0	0.7	99.3	0.0	0.0	0.1	0.0	40.0	0.1	Takamura et al. (1990)
24 h	Algae	<i>Phormidium</i> sp.	7.6	35.0	0.7	99.3	0.0	0.0	0.1	0.0	40.0	0.2	Takamura et al. (1990)
72 h	Algae	Green algae (<i>Pseudokirchneriella subcapitata</i>)	7.3	245.0	0.5	0.3	99.2	17.8	20.2	0.0	109.5	1.2	Heijerick et al. (2005)
72 h	Algae	Green algae (<i>Pseudokirchneriella subcapitata</i>)	5.5	194.0	7.9	0.1	92.0	10.3	2.4	0.0	2.8	15.3	Heijerick et al. (2005)
72 h	Algae	Green algae (<i>Pseudokirchneriella subcapitata</i>)	5.9	152.0	5.4	0.1	94.4	8.9	8.4	0.0	2.9	8.3	Heijerick et al. (2005)
72 h	Algae	Green algae (<i>Pseudokirchneriella subcapitata</i>)	6.3	97.4	19.7	1.1	79.2	2.7	7.0	0.0	4.8	19.2	Heijerick et al. (2005)
72 h	Algae	Green algae (<i>Pseudokirchneriella subcapitata</i>)	7.6	113.0	0.9	0.8	98.2	6.1	33.0	0.0	48.0	1.1	Heijerick et al. (2005)
72 h	Algae	Green algae (<i>Pseudokirchneriella subcapitata</i>)	8.2	52.7	0.3	1.1	98.6	2.4	24.8	0.0	9.6	0.2	Heijerick et al. (2005)
72 h	Algae	Green algae (<i>Pseudokirchneriella subcapitata</i>)	8.1	33.9	0.5	1.4	98.1	2.0	41.5	0.0	38.4	0.2	Heijerick et al. (2005)
7 days	Fish	<i>Pimephales promelas</i>	8.2	34.1	2.2	9.3	88.6	1.0	1.2	0.0	3.4	0.7	Erickson et al. (1996)
7 days	Fish	<i>Pimephales promelas</i>	8.1	41.7	3.5	12.9	83.6	1.0	1.2	0.0	3.4	1.5	Erickson et al. (1996)
7 days	Fish	<i>Pimephales promelas</i>	8.8	56.8	0.7	22.9	76.4	1.0	1.2	0.0	3.4	0.4	Erickson et al. (1996)
7 days	Fish	<i>Pimephales promelas</i>	7.4	14.4	4.5	2.9	92.6	1.0	1.2	0.0	3.4	0.6	Erickson et al. (1996)
7 days	Fish	<i>Pimephales promelas</i>	6.5	5.4	9.0	0.8	90.2	1.0	1.2	0.0	3.4	0.5	Erickson et al. (1996)
7 days	Fish	<i>Pimephales promelas</i>	8.2	52.0	4.2	17.9	77.9	1.0	1.2	0.0	3.4	2.2	Erickson et al. (1996)
7 days	Fish	<i>Pimephales promelas</i>	8.1	176.1	12.1	49.2	38.7	6.0	1.2	0.0	3.4	21.3	Erickson et al. (1996)
7 days	Fish	<i>Pimephales promelas</i>	8.1	133.8	11.9	41.4	46.7	3.5	1.2	0.0	3.4	16.0	Erickson et al. (1996)
7 days	Fish	<i>Pimephales promelas</i>	8.1	78.2	7.4	28.2	64.4	2.3	1.2	0.0	3.4	5.8	Erickson et al. (1996)
7 days	Fish	<i>Pimephales promelas</i>	8.0	42.2	4.7	13.0	82.2	1.0	1.2	0.0	3.4	2.0	Erickson et al. (1996)
7 days	Fish	<i>Pimephales promelas</i>	8.2	11.2	0.3	1.1	98.6	1.0	1.2	0.0	3.4	0.01	Erickson et al. (1996)
7 days	Fish	<i>Pimephales promelas</i>	8.1	11.6	0.3	1.3	98.4	1.0	1.2	0.0	3.4	0.01	Erickson et al. (1996)
7 days	Fish	<i>Pimephales promelas</i>	8.8	16.4	0.0	1.5	98.4	1.0	1.2	0.0	3.4	0.01	Erickson et al. (1996)
7 days	Fish	<i>Pimephales promelas</i>	7.4	8.0	1.9	1.2	96.8	1.0	1.2	0.0	3.4	0.2	Erickson et al. (1996)
7 days	Fish	<i>Pimephales promelas</i>	6.5	5.9	9.8	18.9	71.3	1.0	1.2	0.0	3.4	0.6	Erickson et al. (1996)
7 days	Fish	<i>Pimephales promelas</i>	8.2	17.5	0.6	2.7	96.7	1.0	1.2	0.0	3.4	0.1	Erickson et al. (1996)
7 days	Fish	<i>Pimephales promelas</i>	8.1	15.8	0.6	2.3	97.2	1.0	1.2	0.0	3.4	0.1	Erickson et al. (1996)
7 days	Fish	<i>Pimephales promelas</i>	8.1	117.4	10.0	39.2	50.8	6.0	1.2	0.0	3.4	11.7	Erickson et al. (1996)
7 days	Fish	<i>Pimephales promelas</i>	8.1	61.8	6.5	21.7	71.9	3.5	1.2	0.0	3.4	4.0	Erickson et al. (1996)
7 days	Fish	<i>Pimephales promelas</i>	8.1	31.3	2.1	8.1	89.8	2.3	1.2	0.0	3.4	0.7	Erickson et al. (1996)
7 days	Fish	<i>Pimephales promelas</i>	8.0	13.2	0.7	1.8	97.5	1.0	1.2	0.0	3.4	0.1	Erickson et al. (1996)

Major cations is not included in the table, but included in the speciation calculations performed with the model Visual Minteq version 2.60

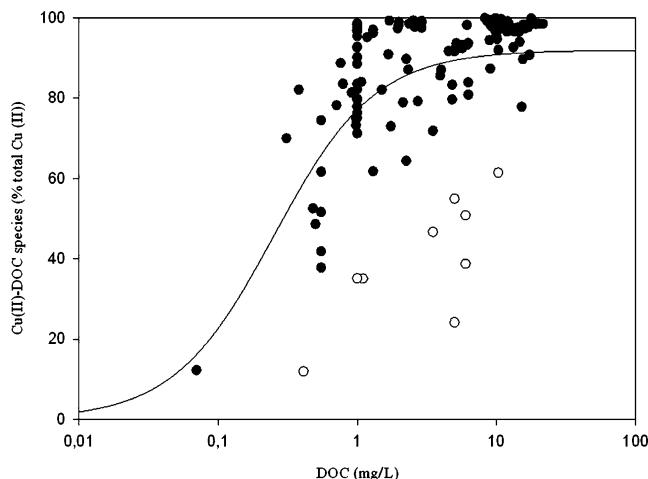


Fig. 1 Relationship between DOC concentrations (mg/L) in the media and the calculated fraction of Cu(II)-DOC species relative to the total dissolved Cu concentration in the system. Nine outliers are identified and marked by open circles. They may be explained by a high content of CO_3^{2-} in the media competing with DOC or Al^{3+} and Fe^{2+} competing with Cu^{2+} for the DOC binding sites. The curve is a guide to the eye as best fit created by SigmaPlot version 11.0

indicated an active role of organic or inorganic complexants on uptake of copper and other toxic metals by fish and plants, and another study suggests that DOC complexes in media limit the uptake and incorporation of copper in daphnids (Bossuyt et al. 2005).

Figure 4 shows the relationship between the calculated free Cu^{2+} ion concentration and the reported acute and chronic EC_{50} values for *D. magna*. In both cases, a strong vertical trend is observed with all calculated concentrations of free Cu^{2+} ions within the range of 0.01–16.8 $\mu\text{g/L}$ for acute studies and 0.01–10.5 $\mu\text{g/L}$ for chronic studies, while

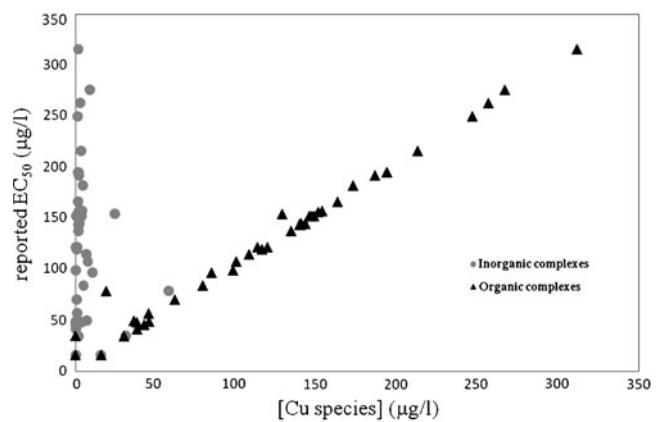


Fig. 3 Reported EC_{50} values for chronic exposure of *D. magna* to Cu shown as function of the calculated concentration of Cu in the form of inorganic or organic complexes in the applied media

the reported EC_{50} values vary between 6.7 and 638 $\mu\text{g/L}$. As water chemistry has been accounted for in the calculation, the broad ranges merely reflect experimental variations between different studies in combination with uncertainties about DOC composition and reactivity. In fact, these ranges may be narrower as closer inspection of Table 1 shows that only few $\text{EC}_{50} [\text{Cu}^{2+}]$ exceed 5 $\mu\text{g/L}$ and the high values are mainly found in chloride-rich and/or acidic media (data not shown) indicating reduced Cu^{2+} toxicity to *D. magna* in such media. Furthermore, competition between toxic Cu^{2+} and nontoxic cations such as Ca^{2+} at active sites (biological ligands) on test organisms may seriously contribute to the rather broad EC_{50} range as indicated by Meyer (1999) and Koster et al. (2006)

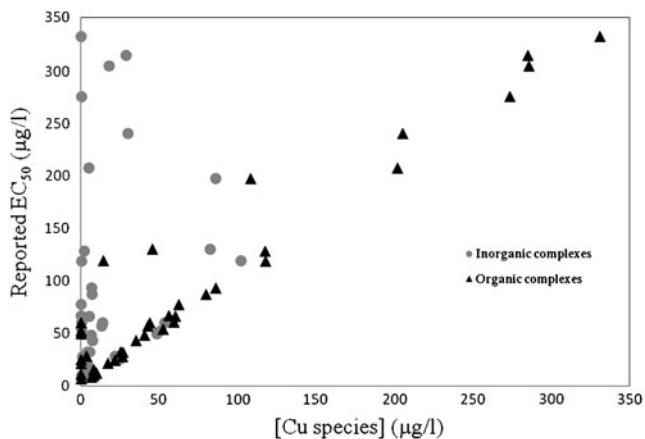


Fig. 2 Reported EC_{50} values for acute exposure of *D. magna* to Cu shown as function of the calculated concentration of Cu in the form of inorganic or organic complexes in the applied media

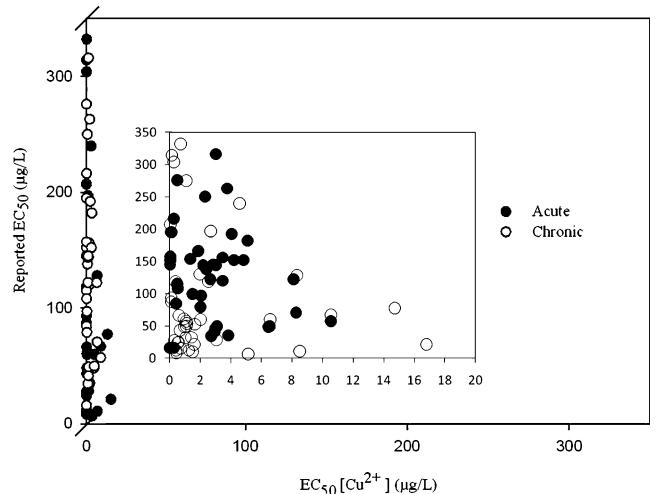


Fig. 4 Reported EC_{50} values for acute (filled circles) and chronic (open circles) exposure of *D. magna* to Cu shown as function of the calculated free $[\text{Cu}^{2+}]$ ion concentration in the applied media. A close-up to show the range of $\text{EC}_{50} (\text{Cu}^{2+})$ from 0.01–16.8 $\mu\text{g/L}$

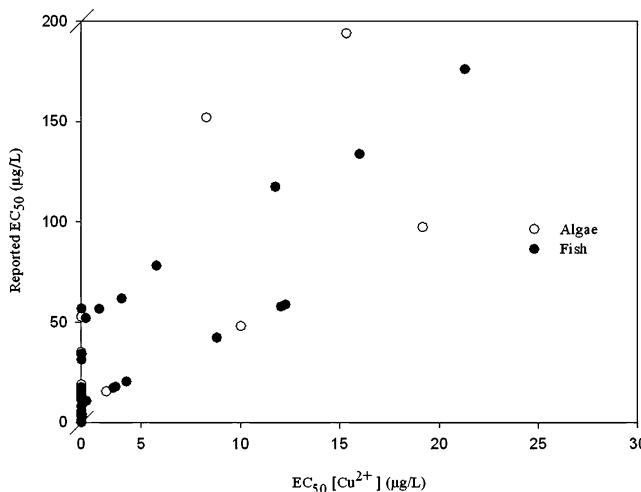


Fig. 5 Reported EC₅₀ values for chronic exposure of algae (open circles) and fish (filled circles) to Cu shown as function of the calculated free [Cu²⁺] ion concentration in the applied media

Figure 5 shows the similar relationship for chronic studies of different algae and fish species. The variation is larger than observed for *D. magna* in Fig. 3, but this may be attributed to the fact that the data represent several different species of both fish and algae.

3.3 Estimation of free ion EFs

Table 3 presents HC₅₀ values calculated as geometric means of reported EC₅₀ values and of the modelled free Cu²⁺ ion EC₅₀ values for each of the phyla crustaceans, algae and fish. As basis of the EF (Eq. 2), the geometric mean is calculated in two ways; first the geometric mean is calculated for EC₅₀ values reported for each species. Then the geometric mean is calculated across all species results (GM_{all}) or across the geometric means of each of the three phyla (GM_{troph}).

The lowest phylum HC₅₀ calculated as the GM of reported EC₅₀ values is found for fish. Taking Cu speciation into account, the difference between phyla is modest and still the lowest HC₅₀ as GM of EC₅₀ [Cu²⁺] is for the phylum fish followed by algae. The geometric mean of the reported EC₅₀ values across all test organisms, GM_{all}, is 65.2 µg/L and shows no real difference from the geometric mean across the means for each of the three phyla crustaceans, algae and fish, GM_{troph}, (63.6 µg/L). For the free Cu²⁺ ion the two different ways of calculating the HC₅₀ value also gives similar result (0.90 µg/L for GM_{all} and 0.84 µg/L for GM_{troph}). The HC₅₀ drops by two orders of magnitude between total dissolved and free Cu²⁺ ion concentration.

For *D. magna* also the HC₅₀ based on acute EC₅₀ values was calculated. For reported EC₅₀ values, it was 58.0 µg/L

Table 3 HC₅₀ as GM of reported chronic EC₅₀ values and calculated free ion EC₅₀ values for each of the phyla, crustacean (*D. magna*), algae and fish

	<i>D. magna</i> (µg/L)	Algae (µg/L)	Fish (µg/L)	GM _{all} (µg/L)	GM _{troph} (µg/L)
No speciation, total dissolved Cu					
HC ₅₀	105.40	56.57	28.73	65.18	63.58
EF				0.008	0.008
Speciation, free Cu ²⁺ ion					
HC ₅₀	1.17	0.73	0.62	0.90	0.84
EF				0.56	0.60

On this basis, HC₅₀ and the corresponding effect factor (EF) are calculated across all test organisms (GM_{all}) and across the GMs of the three phyla (GM_{troph})

calculated as geometric mean, and for the modelled free Cu²⁺ ion EC₅₀ values, it was 0.85 µg/L. There was no statistically significant difference between the geometric means for acute and chronic EC₅₀ values, neither for the reported values, nor for the calculated free Cu²⁺ ion EC₅₀ values.

4 Discussion

4.1 Influence of media characteristics on Cu-toxicity

A comprehensive review of studies reporting ecotoxicity of Cu to *D. magna*, fish and algae (Barata et al. 1998; Biesinger and Christensen 1972; Blaylock et al. 1985; Bossuyt and Janssen 2003; De Schampelaere and Janssen 2002, 2004a, b; De Schampelaere et al. 2004, 2005; Enserink et al. 1991; Hickey and Vickers 1992; Koivisto et al. 1992; Kramer et al. 2004; Leblanc 1982; Muyssen and Janssen 2007; Vanleeuwen et al. 1988; Villavicencio et al. 2005) revealed a dispersion of measured effect concentrations between 6 and 638 µg/L among the studies without any obvious trend (see Tables 1 and 2). When speciation modelling was performed and the free Cu²⁺ ion concentration calculated for all the studies, it was found as shown in Fig. 4 that the same free Cu²⁺ ion concentration may correspond to many different reported EC₅₀ values due to the different media composition applied in different studies, but all the reported EC₅₀ values correspond to a free Cu²⁺ ion concentration between 0.01 and 16 µg/L. This observation indicates that the free ion seems to represent the toxic forms of Cu better than the total Cu. The scattering of points around the vertical line could be taken as indication of experimental uncertainty, uncertainties or errors in the applied speciation modelling, and of the

toxicity of less dominant Cu species which is not accounted for here.

4.2 The importance of DOC and inorganic complexes for the toxicity of Cu

It is well known that DOM such as humic and fulvic acids naturally present in aquatic environments has affinity to bind Cu, by the formation of strong Cu-DOM complexes, see e.g. Meador (1991) and Kim et al. (1999). Studies based on laboratory experiments demonstrate that toxicity to *D. magna* is reduced by humic matter or DOC (Meador 1991; Paulauskis and Winner 1988). The results gathered here across a large selection of studies documents that DOC has a major impact on the toxicity of dissolved Cu as illustrated in Figs. 2 and 3, where more DOC in the media is found to correspond to a higher reported EC₅₀ for *D. magna*. Similar results were found by Bossuyt and Janssen (2003), reporting a linear relationship between EC₅₀ and the calculated concentration of Cu bound to DOC, and by Kramer et al. (2004), who tested Cu toxicity to *D. magna* in six different natural waters and showed that an increase in DOC increased EC₅₀ and decreased the acute toxicity of Cu as also observed in Fig. 2.

While the link between acute Cu toxicity and DOC has been documented by several authors, it has rarely been done for chronic studies (De Schampelaere and Janssen 2004b). Figure 1 indicates that for media with more than 1 mg/L DOC, 70% or more of the Cu is predicted to be bound to DOC, and the bioavailability of Cu reduced accordingly. Similar results have been observed in exposure of *Pseudomonas fluorescens* bacteria as a biosensor by Nybroe et al. (2008) where nearly all Cu in the media is found as Cu-DOM complexes when the concentration of DOC exceeds 1 mg/L. Assuming that the DOC in the test media behaves like NICA-Donnan DOC, the implications of these findings are that the Cu-DOM fraction makes up a relatively constant and dominating fraction of the total dissolved Cu for all test media in this study. In comparison to organic complexes, the inorganic complexes seem to play a limited role in mitigating the ecotoxic effect of Cu. The results for the inorganic complexes (see Figs. 2 and 3) show a vertical pattern indicating that the inorganic complexes can be toxic, maybe because they are being redistributed into free Cu²⁺ ion in accordance with their relatively low stability constants (Lee et al. 2005). Assuming that the Cu hydroxy complexes are the most labile inorganic complexes, it was tested whether the inclusion of this fraction of Cu complexes into the free Cu²⁺ fraction significantly changed the pattern as observed in Figs. 2 and 3, but it was found not to be the case (data not shown). As indicated by the figures (and Table 1), the concentration of the inorganic Cu complexes is small,

which will limit influence of these Cu complexes. However, as indicated above, competition between Cu²⁺ and Ca²⁺ and other nontoxic cation at active organism sites (biotic ligands) as well as for DOC may seriously affect the results.

4.3 Chronic and acute toxicity

EC₅₀ has been calculated for both chronic (129.3±72.7) and acute (102.4±124.5) toxicity to *D. magna*. Contrary to what might be expected, reported acute EC₅₀ values are not higher than the reported chronic EC₅₀ values, and the same holds true for the calculated EC₅₀ [Cu²⁺] values (see Table 1). This indicates that for Cu toxicity, it is not the duration of exposure but the endpoint that is important. All of the reported acute EC₅₀ values are based on immobilisation of the test organism, while chronic values mainly are based on reproduction. A former study of the toxicity of chromium (VI) showed that also for this metal, the test duration had no effect on the toxicity (Birkved and Payet 2003). The standard deviation is very high for both acute and chronic toxicity giving no statistically significant difference between the two.

4.4 Implications and influence of speciation on EFs for LCIA

Recent recommendations (Diamond et al. 2010; Gandhi et al. 2010, 2011) on metal hazard assessment within LCA suggest introducing a bioavailability factor to improve the ability of the fate part of the CF to reflect the exposure of aquatic organisms to metals. The proposed bioavailability factor, BF, translates the total dissolved metal represented by the FF into the bioavailable fraction which quantifies the fraction of total dissolved chemical that is truly dissolved, assuming the latter is equivalent to the bioavailable fraction (Diamond et al. 2010) and changes the expression of the characterisation factor to

$$CF_{j,i,s} = BF_{i,s} \times FF_{j,i,s} \times EF_{i,s} \quad (3)$$

For consistency in the calculation of the characterisation factor for metals according to Eq. 3, it is important that the effect factor also is expressed as bioavailable metal rather than as total dissolved metal, and the proposed EF based on free ion Cu²⁺ concentrations fulfils this requirement.

The results presented in Table 3, show that when speciation is taken into account, there is little difference in HC₅₀ across the three phyla (0.66–1.2 µg/L), and the most sensitive species shifts from fish to algae when we go from reported EC₅₀ values to free Cu²⁺ ion values. Furthermore, the results of Table 3 document that the ratio in toxicity for the phyla is reduced from 3.6 to 1.9 when Cu speciation is taken into account, and by introducing the BF the FF is made consistent with the speciation-corrected EF, and the

influence of differences in test media is reduced. EF will be reduced by two orders of magnitude for both GM_{all} and GM_{troph} when speciation is accounted for (see Table 3).

The determination of EF based on geochemical speciation calculations involves assumptions about the behaviour of the DOC in the test media, which may cause uncertainty to the result. The model assumes DOC of the different media to be the same. Together with the potentially large experimental uncertainty in determining the concentration of DOC in waters from different sites and seasons (Hruska et al. 2003), the natural differences in DOC could explain the range of effect concentrations calculated for the free Cu^{2+} ion.

5 Conclusions, recommendations and perspectives

The use of the calculated free Cu^{2+} concentration in the test media as alternative to total dissolved Cu concentration minimises the variation for the Cu EC₅₀ values for all reported chronic studies. Although some scatter is observed, possibly related to the data points from different sources and DOC of various origins, the results confirm our hypothesis, that the acute and chronic Cu toxicity expressed by EC₅₀ for *D. magna*, fish and algae is mainly controlled by the concentration of the free Cu^{2+} ion. This means that when including the bioavailability in the calculation of metal characterisation factors, the relevant EF could be based on the free ion concentration leading to 50% of the species becoming affected. Using speciation models to determine the free ion concentration in the test media, the resulting changes in the EF amount to around two orders of magnitude increase for Cu. Whether the EF is determined from the geometric mean across all test organisms or across the geometric means for each of the three trophic levels has no influence on the result for Cu.

The free ion concentration seems preferable to other measures of bioavailability of metals, such as the concentrations determined using the biotic ligand model, BLM since the free ion concentration can be determined for all metals in water and hence allows an equal treatment of all metals encountered in the life cycle inventory, while the BLM, as mentioned before, only is available for a limited number of metals and test organisms.

It is foreseeable that the inclusion of speciation in the characterisation modelling for metals will improve the environmental relevance of the characterisation factors and hopefully give a more correct expression of the ecotoxicity of metals relative to organic compounds. Whether consideration of metal speciation will lead to higher or lower characterisation factors for metals is an open question. The speciation influences both the fate and exposure part (BF·FF) and the effect part (EF) in Eq. 3. The former will be reduced due to the limited fraction of the

metal that is bioavailable, but as observed in this study, the EF will increase when speciation is introduced. The two effects counteract each other, and whether the net result is a CF that goes up or down depends on the speciation properties of the test media, in which toxicity is measured, relative to the speciation properties of the natural waters where the bioavailability is modelled.

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